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The N: P: Si Stoichiometry and Relative Abundance of Diatoms in Ganga River

Jitendra Pandey[1*], Amita Yadav[1] and Rachna Singh[1]

[1]Ganga River Ecology Research Laboratory, Environmental Science Division, Centre of Advanced Study in Botany, Department of Botany, Banaras Hindu University, Varanasi, 221005, India.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

In order to assess the changing pattern of N: P: Si stoichiometry and associated shift in the abundance of diatoms, we analysed DO, BOD, NO_3^-, PO_4^{3-}, DOC and DSi in a 37 km long stretch of Ganga River. The N: P stoichiometry declined (from 13.6 to 5.3) at downstream sites receiving urban influence. Similar was the trend for Si: P ratio indicating relatively higher input of P in comparison to N and Si. The chlorophyll **a** biomass and gross primary productivity (GPP) showed positive correlation with BSi ($P < 0.001$) and P enrichment at downstream favoured the abundance of *Amphipleura, Aulacoseira, Craticula, Cymbella, Fallacia* and *Fragilaria.* Since the excessive growth of exploitative species may replace less adapted diatoms, the skewed elemental stoichiometry in the study river may alter the diatom assemblages with increased abundance of P favoured species at urban sites. The study has relevance addressing diatom community level cause-effect relationships and provides important cues for designing action plan towards restoration of this major river system.

Keywords: Ganga River; diatom; biogenic silica; nutrient stoichiometry.

**Corresponding author: E-mail: jiten_pandey@rediffmail.com;*

1. INTRODUCTION

Diatoms are the largest contributor of biosilicification and a major contributor (over 20%) of global primary production and biological carbon pump [1,2]. The increased density resulting from silicification increases the sinking rate of cells and consequently rapid sequestration of carbon to bottom sediment. Since the biogeochemical cycle of carbon is intricately linked with the biogeochemical cycles of other elements, especially that of nitrogen (N), phosphorus (P) and silicon (Si) [3,4], the N: P: Si elemental stoichiometry become a central tenet in aquatic biogeochemistry and used to link a variety of ecosystem processes including phytoplankton productivity and biological carbon pump [4]. Unlike oceans, the source of silica to river is almost constant due to continuous weathering and sedimentary flow. However, from the last few decades anthropogenic perturbations have altered the elemental stoichiometric ratios of C: N: P: Si from their canonical ratios (106: 16: 1: 16) and subsequently the pattern of nutrient limitation in aquatic ecosystems. Diatoms are unique among phytoplankton in that they require Si in the form of silicic acid [Si $(OH)_4$] for growth and production of their delicate frustules.

The Ganges together with Brahmaputra–Meghna river system with total drainage area of 1.75 million km^2 is second only to Amazon with respect to water discharge. The Ganga River carries the highest silt load of any river in the world and the deposition of these materials results in the largest river delta in the world (400 km from north to south and 320 km from east to west) [5]. The Indo-Gangatic plains constitute the most densely populated region and one of the largest ground water repositories on the earth. During the last two decades the population throughout the Ganges basin has increased massively and the region witnessed unprecedented urban-industrial growth during this period. As a result, the Ganges basin receives massive fluxes of nutrients and other pollutants along its 2525 km course from Gangotri in Himalaya to its confluence with Bay of Bengal. The alarming population growth, unplanned urbanization and industrialization in the plains have become the cause of concern for increasing level of air, soil and water pollution in the Ganges basin [6,7]. Recent studies have indicated that nutrients such as N and P in the river are increasing at alarming rates [7,8], causing a shift in the stoichiometric ratios of critical elements [7,9]. In a more recent study, it has been reported that the increasing atmospheric input of N and P has led to a shift in the pattern of ecological nutrient limitation in Ganga River [9].

Diatoms are used extensively in environmental assessment and monitoring because they have range of tolerance to environmental conditions and show predictable response to a causal factor. Ecological shifts from diatoms to non-siliceous phytoplankton have often been related to shift in nutrient status and elemental stoichiometry [10-12]. Diatoms often serve as indicator of causal relationships in aquatic ecosystems. They show wide range of tolerance along environmental gradients and recover more rapidly from silica starvation than nitrogen deprived conditions upon supply of nutrients [1,2]. Considerable work has been done on nutrient limitation of marine and fresh water phytoplankton [13,14]. Most of the studies on diatoms of Ganga River are based either on presence- absence data or on the analysis of diatom assemblages in samples collected across salinity gradient in salt affected zones and estuaries towards the river confluence with Bay of Bengal [15-17]. Characteristic features of the river such as climatic and topographic diversity, land use pattern in the watershed and a gradient of anthropogenic inputs and atmospheric deposition create wide range of habitat heterogeneity and niche partitioning to support high diversity of riverine primary producers including diatoms. Thus, a clear understanding of small- to large- scale differences in diatom assemblages along the river gradient are essentially required for biomonitoring and water quality assessment of the Ganga River. In particular, information on the effect of varying nutrient concentrations and their stoichiometric ratios on the distribution of diatom assemblage in Ganga River are very scarce. The present work is an effort to investigate the distribution of diatom assemblage as influenced by elemental stoichiomentries along **a** 37 km long gradient of the Ganga River. Chlorophyll a biomass and biogenic silica was used to explore relative contribution of siliceous phytoplankton to overall trophic status of the river.

2. MATERIALS AND METHODS

2.1 Study Area

This two year study was conducted during March, 2012 to February, 2014 (hereafter referred as year 2012 and 2013) at five study sites along a 37 km stretch of the Ganga River covering upstream to downstream urban core of Varanasi city (25° 18' N lat. and 83° 1' E long.). The study area lies in the middle stretch of the Ganges basin, the 4th largest (1, 086, 000 km^2 total area) trans-boundary river basin in the world. For the purpose of the present study, five sites were selected on the basis of input from point and non-point sources. Sites 1 and 2 were relatively under natural control and rest of the sites were invariably human disturbed (Fig. 1). Climate of the region is tropical with three distinct seasons; hot and dry summer (April to mid-June), warm and wet rainy season (mid-June to September) and a cool and dry winter (November to February). October and March represent the transition months. In summer, the temperature sometimes exceeds 46°C. More than 90% of the average annual rainfall (1050 mm) occurs in rainy season. Wind direction shifts from predominantly westerly to south westerly in October to April and easterly to north westerly in remaining months. The soil in the region is highly fertile alluvial fluvisol associated with recurrent floods or long wetness, recent sedimentation and variable proportion of silt and clays.

2.2 Sampling and Analysis

Triplicate samples were collected from 25-30 m reach of the river directly below the surface (15-25 cm depth) in acid rinsed 5L plastic containers. Dissolved organic carbon (DOC), the fraction of organic carbon filtered through 0.7 µm mesh filter, in water samples was estimated using $KMnO_4$ digestion procedure [18]. Nitrate was estimated using phenol disulphonic acid method and NH_4^+ following phenate method [19]. Dissolved reactive phosphorous (DRP) was estimated following ammonium molybdate-stannous chloride method and dissolved silica (DSi) following molybdate-blue method [20]. For biogenic silica (BSi) in sediment, samples

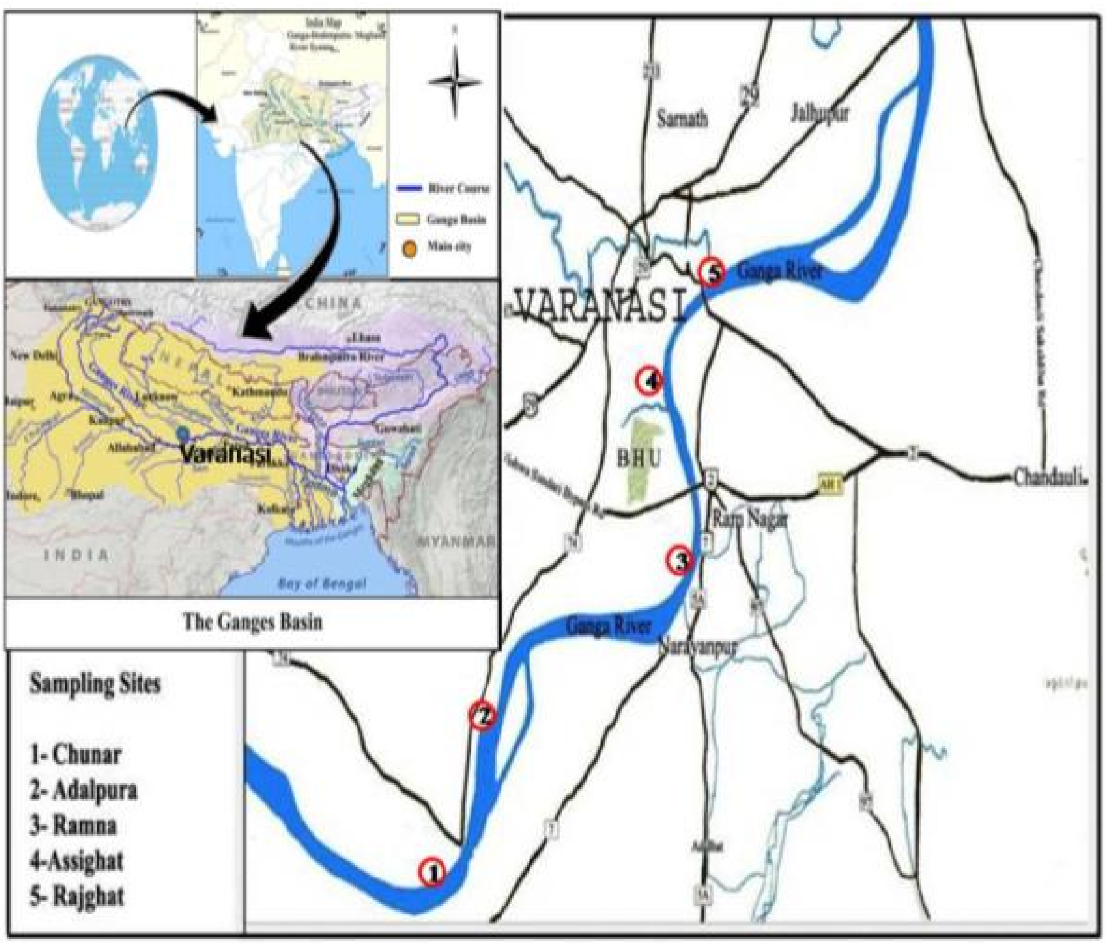

Fig. 1. Ganga River basin showing location of sampling sites

(0-10 cm) were collected from river bottom at 25-30 m reach. One gram of air dried sample (< 2mm) was digested in 40ml of 0.1% Na_2CO_3 and the BSi in digested sample was determined following molybdate blue method [21]. Biological oxygen demand (BOD) and dissolved oxygen (DO) was determined following standard methods [22]. Chlorophyll **a** (Chl **a**) was measured following acetone extraction procedure [23] and gross primary productivity (GPP) was determined by light and dark bottle method [22]. Taxonomic identification of diatoms was made following [24]. The relative abundance of diatom was determined following the method described in [25].

To assess the range of variability, means were supported by standard deviation (SD). Correlation analyses and regression models were employed to test the linearity in relationships. Principal component analysis (PCA) and canonical correspondence analysis (CCA) were used to ordinate sampling locations and environmental variable- linked diatom assemblages.

3. RESULTS AND DISCUSSION

Biological oxygen demand (BOD) and the concentrations of nutrients, DOC and DSi in river increased along the study gradient (Fig. 2). With few exceptions, the values were generally higher in 2013 relative to 2012. Dissolved oxygen (DO) did show an opposite trend. Seasonally, BOD was found to be lowest in monsoon and highest in summer (Fig. 2). Concentrations of NO_3^-, NH_4^+ and PO_4^{3-} were lowest in monsoon and highest in winter. River flow regulates dilution effect and explains seasonal variations [26]. High concentration of nutrients in dry season could be linked to reduced river flow and enhanced input through atmospheric deposition [7]. Dissloved organic carbon (DOC) however, was found highest in monsoon due to the enhanced hydrological flushing of terrestrial carbon. Dissolved silica also was found highest in rainy season due to increased weathering and surface runoff mediated effects [27]. Productivity variables (Chl **a** biomass and GPP) and BSi were lowest in monsoon season. On spatial scale, dissolved oxygen declined down the river gradient with mean values being highest (5.6 mg l^{-1}) at Site 1 and lowest (3.71 mg l^{-1}) at Site 5. On the other hand, BOD and the concentrations of NO_3^-, NH_4^+ and PO_4^{3-} and DOC increased down the gradient. The BOD varied between 3.71 mg l^{-1} and 10.86 mg l^{-1} with values being lowest at Site 1 and highest at Site 5. Concentration of NO_3^- (256.86 - 354.27 µg l^{-1}), NH_4^+ (58.88 - 94.75 µg l^{-1}) and PO_4^{3-} (23.88 - 90.46 µg l^{-1}) showed a similar trend. Chl **a** and GPP showed synchrony with the concentration of nutrients. The BSi (47.9 - 95.8 µg l^{-1}) also followed a spatial trend similar to the nutrients and DOC. The DSi however, did not show significant change and the values ranged between 348.1 and 383.9 µg l^{-1}. Most of the variables showed significant effect of site, year and their interactions (ANOVA) (Table 1).

Table 1. F-ratios from two way analysis of variance (ANOVA) indicating significant effects of year, site and their interactions on different variables of Ganga River

Variables	Year	Site	Year x site
Nitrate	226.09	1372.78	62.34
Ammonia	863.77	7037.74	1752.62
Phosphate	763.45	34189.98	31.72
DO	118.88	4427.49	117.14
DOC	305.54	577.68	69.52
BOD	2030.99	10129.66	72.83
DSi	4022.98	2548.71	850.61
BSi	854.42	5966.18	27.36
Chl **a**	218.21	5396.18	6.80
GPP	14.85	611.96	2.22^{NS}

Values, except marked otherwise, are significant at P < 0.01; NS: not significant

Data on spatio- temporal trends on stoichiometric ratio of nutrients are given in Fig. 3. The N: P declined down the river from 13.5 at Chunar to 5.3 at Rajghat. The Si: P ratio also followed a similar trend. Unlike N: P and Si: P, the N: Si and C: Si ratios increased down the gradient with values ranging between 1.1 and 1.5 for N: Si and, 11.7 and 25.1for C: Si. Year wise, the values did not differ significantly.

The C: N ratio also declined down the river. Declining N: P stoichiometry down the river gradient indicate high input of P relative to N and has relevance since the relative availability of nutrients in an ecosystem determines which nutrient to become a limiting factor [28]. It is expected that the influence of strong local control especially sewage input and high atmospheric deposition could enhance P input proportionately greater than N at downstream sites [7]. Diatoms form an important component of aquatic food chains and require distinct stoichiometric ratio of critical elements (N: P: Si = 16: 1: 16) for their balanced growth. A deviation from these ratios in the environment may shift structure and composition of phytoplankton community

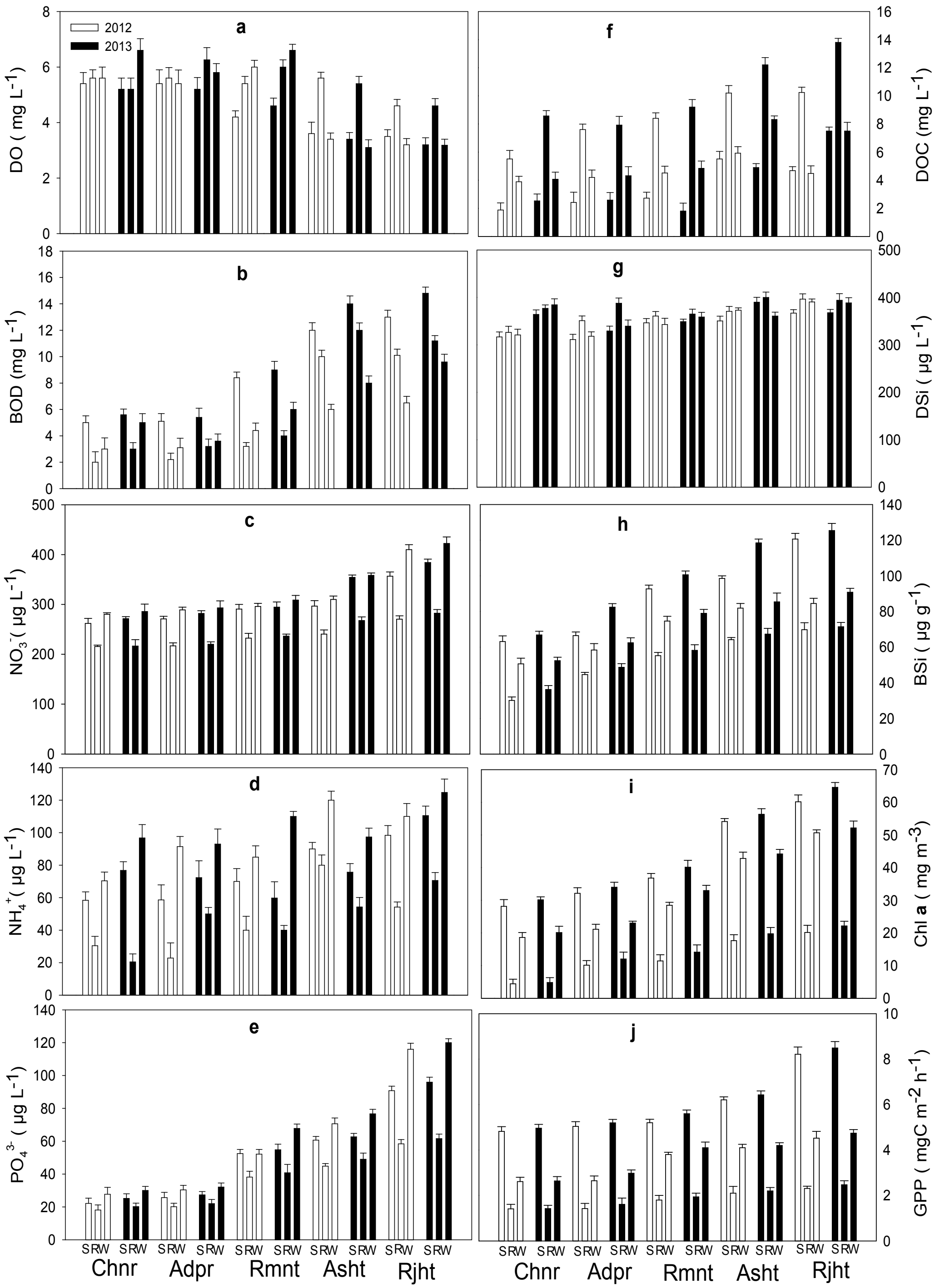

Fig. 2. Spatio - temporal trends in dissolved oxygen (DO, a), biological oxygen demand (BOD, b), NO_3^- (c), NH_4^+ (d), PO_4^{3-} (e), dissolved organic carbon (DOC, f), dissolved silica (DSi, g), biogenic silica (BSi, h), chlorophyll a biomass (Chl a, i) and gross primary productivity (GPP, j) at different study sites of Ganga River. Values are mean (n=24) ± SD

including diatom assemblage. Diatom use Si for cell wall and its availability in the ecosystem control the growth of diatom assemblage [29]. For instance, if the concentration of DSi is less (low Si: N ratio) the community is slowly replaced by non siliceous groups [12]. Thus, the long term nutrient loading and shift in canonical stoichiometry may change the primary producers with an associated shift in trophic cascade. The diatom communities in river are controlled also by discharge rates, for example as regulated by damming and seasonal influences [30,26]. During low flow periods or damming induced changes in flow rate, an increase in nutrients (N and P) and a decrease in DSi [31] may result in the development of flagellated blooms [30]. In this study although N: Si declined along the study gradient, the ratios remained below 1.5 indicating that Si could not be considered as a limiting nutrient in the study stretch. Increased P supply and thereby a shift in N: P stoichiometry may lead to shift diatom population towards a P favoured community [32]. In the present study, the N: P: Si ratios were 14: 1: 13 at Chunar which declined downstream and become 5: 1: 4 at Rajghat indicating strong urban influence as a possible causal factor to shift diatom assemblage in the study stretch.

Our data on the trophic status and concentration of nutrients, DOC, BOD and BSi show a clear gradient from relatively unpolluted upstream to highly polluted urban downstream sites. Principal component analysis (PCA) of the entire data set grouped study sites into four categories along the pollution gradient (Fig. 4).

The PCA segregates Chunar and Adalpura (both as relatively less polluted sites) in one group and Ramna as a separate group with agricultural lands in the catchment. Assighat did appear separately showing still higher pollution load and Rajghat as the most polluted site receiving urban discharge and downstream influences. Since the changes in environmental variables shift diatom species composition [33], an attempt was made to quantitatively link the distribution of major diatom taxa along the pollution gradient. The data on relative abundance showed that *Cocconeis*, *Cyclotella, Hyalodiscus* and *Aulacoseira* were abundant whereas *Diatoma* was the least abundant diatom in the study stretch of the river (Table 2). Species such as *Achnanthidium*, *Amphipleura*, *Asterionella, Craticula*, and *Cyclotella* were found relatively more abundant at highly polluted downstream sites. Such a distribution pattern was marked also, although not explicitly, by canonical correspondence analysis (CCA; Fig. 6). The analysis employed water quality characteristics such as NO_3^-, PO_4^{3-}, DO, BOD, DOC and DSi as environmental variables responsible for structuring the abundance of diatom assemblages along the gradient. The analysis showed that nutrients and DOC were the major determinant of diatom abundance variabilities. The first two axes explained most of the variations in diatom assemblages. Variables such as NO_3^-, PO_4^{3-} and DOC were highly correlated ($P< 0.01$) with the first two axes. Diatom taxa in left upper quadrant consist mainly with *Asterionella, Amphipleura, Cyclotella etc* which are found at sites characterized by high concentration of nutrients and DOC. On the other hand, taxa in lower right quadrant such as *Hyalodiscus*, *Navicula, Nitzschia* etc are favoured by high N: P and Si: P stoichiometric ratios. Genera with higher affinity for nutrients are placed in left upper quadrant. The other part of variation along the study gradient representing the third axis (Fig. 6) can be interpreted as species with intermediate range of nutrient stoichiometric requirement. Thus, the CCA results also show the role of other regulatory variables as well. In earlier studies, abundance of *Aulacoseira* has been linked with high P load [34] and *Pinnularia* with mesotrophic to eutrophic conditions [35]. Unlike this study however, *Synedra* and *Cocconeis* has been linked, although not explicitly, with P-limiting condition [34,36]. As observed in this study, *Achnanthidium* has been reported to grow well in polluted water including those affected by acid mine drainage [37]. Similarly, *Amphipleura and Craticula* which were abundant at downstream urban sites are known to tolerate high nutrient concentration [35,38]. *Diatoma* although known to prefer conductivity range of 100 to 500 µS/cm and withstand mesotrophic to eutrophic conditions, often dominates under elevated P levels [34]. *Gomphonema* which was found distributed almost uniformly at all study sites is reported to tolerate eutrophic to hypereutrophic environment [34]. With minor deviation, the abundance of *Surirella* can be linked to high nutrient and organic load. Some authors have reported *Nitzschia* as a good indicator of heavy metal contamination, eutrophic status and high organic load [39,40]. Almost uniform distribution of *Nitzschia* in this study however, does not support these conclusions. Our previous studies show a marked increase in the concentration of nutrients, heavy metals and organic load downstream the study stretch [41,7]. [42] found abundance of *Synedra* and *Cocconeis* at discharge outlet of pulp and paper mill effluents in Ganga River at Bijnor. In Garhwal region, *Diatoma, Synedra, Gomphonema, Cymbella,* *Nitzschia and Tabellaria* have been reported as dominant species; *Navicula* and *Cyclotella* was major contributor while *Fragilaria* and *Cocconeis* did show occasional appearance [43]. [44] studied shifts in diatom community structure in response to atmospheric deposition of N. These authors observed that *Asterionella* and *Fragilaria* responded strongly to N addition in oligotropic alpine lakes and are indicator of P enrichment in temperate lakes [44]. Our previous studies show atmospheric deposition (AD) as an important source of N and P input to Ganga River [8,7] suggesting AD-input as a possible causal factor for the distribution of diatoms.

Contribution of diatoms to overall trophic status of the river increased at downstream sites. We found strong positive correlation (Fig. 5) of BSi with concentration of nutrients (R^2 = 0.37-0.59; $P < 0.001$), Chl **a** biomass (R^2 = 0.88; $P < 0.001$) and GPP (R^2 = 0.82; $P < 0.001$) indicating that diatom contribution to trophic cascade increased significantly at downstream sites. The BSi was negatively correlated with N: P (R^2 = 0.33; $P <$ 0.001) and Si: P (R^2 = 0.64; $P < 0.001$) indicating the direct influence of increasing P load along the gradient. Increasing BSi down the study gradient could be the result of rapid capitalization of nutrient rich conditions by exploitative diatom species. In some other studies, abundance of *Aulacoseira* and *Diatoma* has been linked with N limiting and high P condition [44]. This could be expected in this study also because urban sewage and biomass burning substantially enhance P loading at downstream sites.

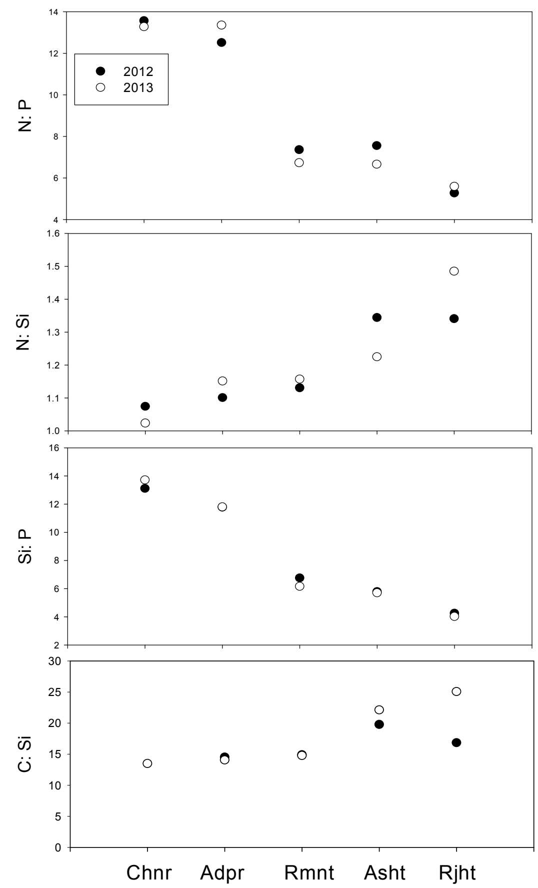

Fig. 3. Stoichiometric ratios of nutrient (N: P, N: Si, Si: P and C: Si) in water at different study sites of Ganga River

Table 2. Spatial variations in relative abundance of diatoms at different study sites in low flow season

Diatom	Code	Relative abundance (%)				
		Chnr	**Adpr**	**Rmnt**	**Asht**	**Rjht**
Achnanthidium	Achn	2.5	6.0	3.1	3.7	4.4
Asterionella	Aste	2.2	2.0	3.2	3.5	3.2
Amphipleura	Amph	2.5	2.6	3.4	4.6	4.4
Aulacoseira	Aula	13.8	11.3	7.1	9.2	10.2
Cocconeis	Cocc	8.6	9.5	8.3	8.9	8.6
Craticula	Crat	2.5	2.0	3.4	2.8	2.9
Cyclotella	Cycl	12.9	10.5	16.7	22.2	20.6
Cymbella	Cymb	2.5	2.0	3.1	2.8	2.2
Diatoma	Diat	1.4	1.7	0.9	1.2	1.5
Fallacia	Fall	2.5	2.0	3.6	2.8	2.2
Fragilaria	Frag	2.5	2.0	3.8	1.8	2.9
Gomphonema	Gomp	2.5	4.0	4.0	1.8	2.2
Hyalodiscus	Hyal	7.6	6.0	4.4	4.6	4.4
Melosira	Melo	7.6	8.0	4.6	6.4	6.5
Navicula	Navi	10.2	12.1	6.5	8.3	8.7
Nitzschia	Nitz	7.6	6.0	4.7	3.7	4.4
Pinnularia	Pinn	2.5	2.0	5.2	2.8	2.2
Surirella	Suri	5.1	6.0	6.2	4.6	4.4
Synedra	Syne	2.5	4.0	7.7	4.6	4.4

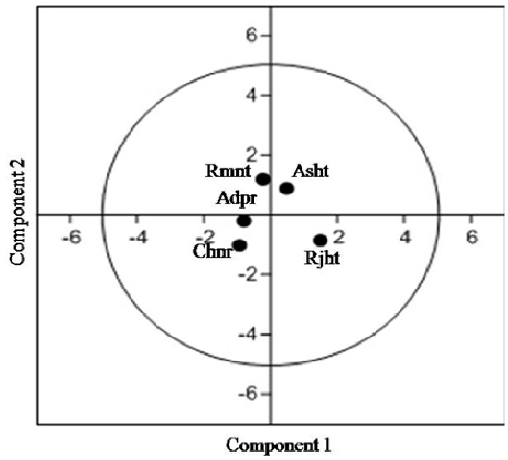

Fig. 4. Principal component analysis (PCA) showing position of sampling locations on different ordinates

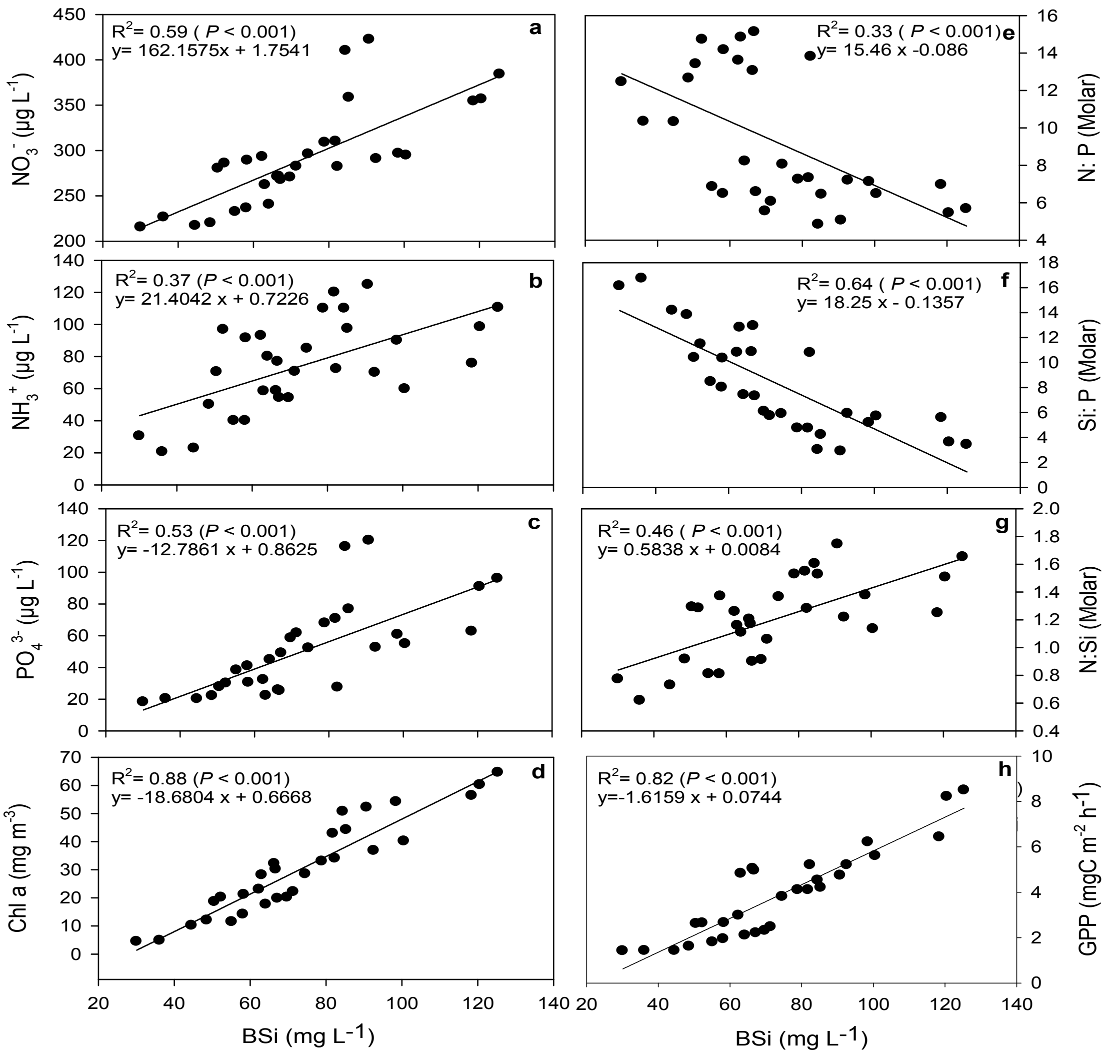

Fig. 5. Correlation of BSi with nutrients and productivity variables

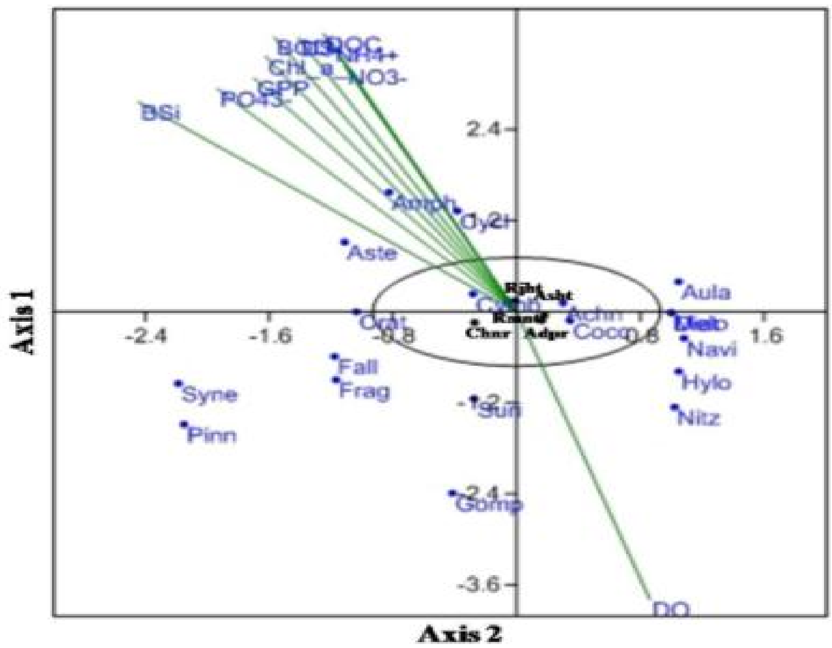

Fig. 6. Canonical correspondence analysis (CCA) ordination biplots of environmental variables and diatom assemblage recorded at five study sites

4. CONCLUSIONS

Correlative evidence in this study suggests that downstream increases in the concentrations of N and P enhance the production of BSi, Chl **a** and GPP. The declining N: P and Si: P ratio also favours the production of BSi. We conclude that although, the distribution of diatoms is regulated by multiple factors, P rich conditions favour the dominance of certain diatom species such as *Amphipleura, Aulacoseira, Craticula, Cymbella, Fallacia and Fragilaria.* Excessive growth of exploitative species may replace less adapted diatom species. Since diatoms play a key role in ecosystem recovery, our study suggests the need for proper planning for management of the watershed and point and non- point sources of nutrient input for the restoration of this major river system. We thank Head, Department of Botany for facilities and Banaras Hindu University for financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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